REMARKS/ARGUMENTS

Applicants respectfully request reconsideration and allowance of this application in view of the amendments above and the following comments.

Applicants have amended the main claims 1 and 27 to incorporate the substance of claims 3 and 7. Claim 30 has been amended to incorporate the substance of claim 7. In addition, Applicants have canceled claims 3 and 7 as being superfluous. Applicants do not believe that any of the amendments introduce new matter. An early notice to that effect is earnestly solicited.

Claims 1-3, 5-24, 26, 27, 29 and 30 were objected to as depending in part on claims that are withdrawn from consideration. In response, Applicants have limited the claims to the elected invention. Applicants expressly reserve the right to prosecute the non-elected subject matter in a divisional application.

Claim 2 was objected to under 37 CFR § 1.75(c) as being an improper dependent claim for failing to further limit the subject matter of claim 1. In response, Applicants have canceled claim 2.

Claim 29 was rejected under 35 USC § 112, second paragraph, as being indefinite. In response, Applicants have amended claim 29 in a manner which Applicants believe overcomes the Examiner's concern.

Claims 1-3, 5-24, 26, 27 and 29 were rejected under 35 USC § 112, first paragraph, as

being broader than the enabling disclosure. In response, Applicants respectfully submit that the amended claims are fairly enabled by the original disclosure.

The limitation to "mouse" as requested by the Examiner is not deemed to be necessary as at the filing date of the present application, namely the end of 2003, a person skilled in the art was well in the position to perform gene knock-down/gene knock-in either randomly or through homologous recombination in any type of animal once it was shown for the first animal. The Examiner concedes enablement for mice. In view of the state of the art, the enablement should be considered reasonably assured for other animals as well.

As to the third issue, Applicants believe it improper to require them to limit to a particular phenotype. The gist of the method of the present invention is to provide a powerful expression system for shRNA in a given animal. Applicants respectfully submit that the invention would be regarded by persons skilled in the art as enabled far beyond the scope the Examiner concedes.

In view of the foregoing, Applicants respectfully request that the Examiner reconsider and withdraw this rejection. An early notice that this rejection has been reconsidered and withdrawn is earnestly solicited.

Claims 27, 29 and 30 were rejected under 35 USC § 102(b) as being anticipated by Buvoli et al. ("Buvoli"), *Mol. Cell Biol., 20:* 3116-24 (2000). In response, Applicants respectfully submit that the amendments to claims 27 and 30 moot this rejection. Buvoli does not teach an expression vector containing homologous sequences which integrate through homologous recombination at a polymerase II dependent locus in the genome of a non-human vertebrate, as required by all of the rejected claims.

Therefore, Applicants respectfully request that the Examiner reconsider and withdraw this rejection as well. An early notice that this rejection has been reconsidered and withdrawn is earnestly solicited.

Claims 1, 2, 5, 7-11, 13, 14, 24, 26, 27 and 29 were rejected under 35 USC § 102(e) as being anticipated by Beach et al. ("Beach"), US 2003/0084471. In response, Applicants point out that claim 3 was not subject to this rejection, and, as noted above, the substance of claim 3 has been incorporated into main claims 1 and 27. Accordingly, all of the rejected claims should now be free of this rejection.

Therefore, Applicants respectfully request that the Examiner reconsider and withdraw this rejection as well. An early notice that this rejection has been reconsidered and withdrawn is earnestly solicited.

Claims 1-3, 5-10, 13-24, 26, 27, 29 and 30 were rejected under 35 USC § 103(a) as being obvious over Beach, Bronson et al. ("Bronson"), *Proc. Natl. Acad. Sci. USA, 93:* 9067-9072 (1996), and Soriano et al. ("Soriano"), US 6,461,864.

Claims 1-3, 5-24, 26, 27, 29 and 30 were rejected under 35 USC § 103(a) as being obvious over Beach, Bronson, Soriano and Ohkawa et al. ("Ohkawa"), *Hum. Gene Ther., 11:* 577-85 (2000).

In response to ***both*** obviousness rejections, Applicants respectfully submit that the cited combination of references does not make out a *prima facie* case of obviousness. Therefore, Applicants respectfully request that the Examiner reconsider and withdraw both obviousness

rejections as well.

Applicants concede that Beach demonstrates that a luciferase specific shRNA under the control of the U6 promoter can mediate widespread gene silencing in cultured cell lines (referred as "*in vivo*" in this document). However, the document lacks any information about shRNA mediated RNAi in a multicellular organism.

Therefore, Beach does not teach, suggest or enable ubiquitous gene silencing via shRNA expression in transgenic animals.

In addition, it is technically not correct that expression of the shRNA transgene implies random integration into a polymerase II rather than a polymerase I or III dependent locus in order to induce luciferase suppression. In fact, Beach did not further analyze transgenic cell lines in respect to the integration site. Therefore, it was not obvious to a person skilled in the art that a polymerase III dependent promoter could exert sufficient activity when integrated into a polymerase II dependent locus.

Furthermore, Beach did not determine the number of shRNA copies integrated into the genome of transgenic cell lines. Usually, random integration of transgenes results in a concatameric array of multiple copies, whereas single copy integrations are unusual (Martin & Whitelaw, *BioAssays 18:* 919-923 (1996)).

Thus, it was questionable whether a single copy of a siRNA expression vector integrated into the genome would result in sufficiently high levels of siRNA required for RNAi-mediated gene inhibition in multiple organs of the living organism.

As the amended claims require that the incorporation is effected by homologous recombination, the claimed animal has only a single incorporation event at the desired locus. This distinguishes the instantly claimed animal from that of Beach.

To overcome problems of random transgenesis, such as a variable level and pattern of expression and lack of germline transmission, Bronson applied homologous recombination to introduce a single transgene copy into the HPRT locus. However, several reports implied that "targeted transgenesis" at the HPRT locus is not suitable to avoid unpredictable position effects in transgenic animals: Insertion of a lacZ gene under the control of the polyoma enhancer/HSV thymidine kinase promoter into Hprt resulted in variable ß-galactosidase expression that was both orientation and cell-type dependent (Shaw-White et al., *Transgenic Res. 1:* 1-13 (1993)). Transgenes under the control of the human and the chicken ß-actin gene promoter showed widespread expression when inserted into the Hprt locus. Unexpectedly, however, the level of transcripts varied strongly in different tissues and expression of these transgenes, in contrast to the endogenous HPRT gene, appeared to be low or undetectable in kidney and liver (Bronson et al., *Proc. Natl. Acad. Sci. USA, 93(17):* 9067-72 (1996)). Hatada et al. demonstrated that the HPRT locus suppresses the activity of both the haptoglobin gene promoter as well as the herpes simplex thymidine kinase promoter in several tissues of mice (Hatada et al., *J. Biol., Chem., 274(2):* 948-55 (1999)). Likewise, a human eNOS promoter-LacZ reporter gene placed in the Hprt locus was found to be inactive in hepatic vessels that otherwise express the endogenous eNOS gene (Guillot et al., *Physiol. Genomics, Mar. 13, (2):* 77-83 (2000). Finally, since the HPRT gene is on the X chromosome, transgene expression at this locus is subjected to random X-inactivation. The expression of the transgene in all cells of the female, therefore, requires the

generation of homozygotes.

Therefore, the combination of Beach and Bronson did not provide motivation of targeting a single copy of a transgenic sequence to a chosen location in the genome such as HPRT for ubiquitous expression.

Soriano describse a method for the production of transgenic animals, which ubiquitously express a heterologous gene inserted into the Rosa26 locus through homologous recombination. In this configuration, the endogenous rosa26 promoter drives transgene expression via a splice acceptor sequence. In contrast, Soriano did not invest activity of exogenous promoters when stably inserted into rosa26.

Therefore, the combination of Beach, Bronson and Soriano did not provide motivation to insert transgenes under the control of an exogenous polymerase III-dependent promoter into rosa26 to achieve ubiquitous expression.

Okhawa does not overcome the above-noted deficiencies in the combination of Beach, Bronson and Soriano.

Considering the foregoing, and taking the various combinations of references as the Examiner has combined them, Applicants respectfully submit that it would not have been *prima facie* obvious for a person skilled in the art that a RNA polymerase II dependent locus such as *rosa26* can promote appropriate expression of a single shRNA construct under the control of a polymerase III dependent promoter required for ubiquitous RNAi in a living organism.

Applicants respectfully submit that both obviousness rejections can be reconsidered and

withdrawn on this basis alone.

However, Applicants also point out that the finding that the shRNA can be expressed in polymerase II dependent loci such as Rosa26 was surprising and unexpected for the following reasons: First, Applicants point out that it is generally known in the art that short intron-free RNA molecules are exclusively expressed at RNA polymerase III dependent loci. The regulatory DNA sequences required for the expression are not known so far. Further, shRNA are generally known to belong to the class of short intron-free RNA molecules. Therefore, it would not have been obvious to a person skilled in the art that shRNA could be expressed in an RNA polymerase II dependent loci such as Rosa26 locus at an efficient rate. Second, it is not derivable from any of the cited art that polymerase dependent promoters are suitable for the expression of shRNA constructs. Above all, it was not obvious that a combination of polymerase II dependent locus with a polymerase promoter as utilized within the invention of the present application would provide for an efficient expression of shRNA construct. In fact, as can be seen from the experimental part of the present application such a polymerase dependent locus in combination with a polymerase-dependent promoter provides for efficient repression of a target gene through expression of an appropriate shRNA construct (see Example 4).

Applicants respectfully request that the Examiner reconsider and withdraw both obviousness rejections. An early notice that both obviousness rejections have been reconsidered and withdrawn is earnestly solicited.

Applicants believe that the foregoing constitutes a bona fide response to all outstanding objections and rejections.

Applicants also believe that this application is in condition for immediate allowance. However, should any issue(s) of a minor nature remain, the Examiner is respectfully requested to telephone the undersigned at telephone number (212) 808-0700 so that the issue(s) might be promptly resolved.

Early and favorable action is earnestly solicited.

Respectfully submitted,
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By _____

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